Neonatal responsiveness to the odor of amniotic fluid

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ABSTRACT
This study evaluated the neonatal responsiveness to the odor of amniotic fluid during the first hours of life. Twenty five newborn infants born by C-section at 36-40 weeks of gestation were evaluated with 1, 5 or 25 drops of amniotic fluid at 1 hour, 3.5 hours and 6 hours of life. A five stimuli habituation plan was used and global motor activity was recorded. Mixed-design ANOVAs for repeated measures demonstrated time differences and showed the highest reactivity at 1 hour, a reduction at 3.5 hours and a recovery at 6 hours (p < 0.0000). Maximum response was observed at the beginning, with a subsequent reduction (p < 0.0008). T tests were used to compare mean differences from baseline to the stimulation period. It was observed that one drop was enough to trigger a response, even at 6 hours of life (p ≤ 0.045). Apparently, babies are able to detect minimum amounts of amniotic fluid and habituate the response with repeated exposure during their first hours of life.

Key words: amniotic fluid, fetal memory, human newborn infant, sense of smell.

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INTRODUCTION
There is some evidence that the structures responsible for detecting odors are functional during the third trimester of gestation. Apparently, the main olfactory system would already have morphologically mature neuronal receptors at the end of the first trimester, which would become functional in the third trimester.1,2

In newborn infants, the olfactory context involves the odor of maternal body and breast milk secretion, but for the fetus, it is the amniotic fluid that triggers the chemosensory cues surrounding it. The neonatal responsiveness to the odor of amniotic fluid has been studied, for example, by soaking one breast with amniotic fluid and observing which breast the baby would suck first,3 or by analyzing how the baby turns the head when breasts are presented with such odor or that of a different woman.4

It seems babies always prefer their own odor.

This study intends to evaluate newborn infants’ responsiveness to the odor of amniotic fluid during their first hours of life by evaluating the minimum dose required to trigger a response, the period such response lasts in their memory and the possibility of habituation after repeated exposure. Habituation involves a process of central information integration with a gradual decrease in response to a repeated and known stimulus. Habituation has been investigated in preterm and term infants by means of vibroacoustic,5 auditory,6 tactile7 and visual8 stimulation. Habituation to odors has been shown in the olfactory system,9 but there is little information regarding such responsiveness to the odor of amniotic fluid and odor detection in small doses, as proposed in this study.

POPULATION AND METHODS
Design
An experimental study was conducted by means of olfactory presentation of different doses of amniotic fluid (1, 5 or 25 drops) at three different times (at 1 hour, at 3.5 hours and at 6 hours of life). Each newborn infant was examined three times with a different dose each time.

Population
Male and female newborn infants born at the Neonatology Division of Universidad Nacional de Córdoba between April 2008 and May 2009. Inclusion criteria were as follows: birth by scheduled C-section; gestational age more than 36 weeks; absence of gesta-
tional, perinatal or neonatal complications, such as fetal distress, hypoxia or respiratory distress syndrome; absence of genetic or chromosomal disorders or maternal diseases that might interfere with a detection and orientation response to the stimuli offered.

Ethical aspects
Only newborn infants whose parents had signed the written informed consent (Health Research Ethics Institutional Boards of the Province of Córdoba, Health Research Provincial Registry No. 1629) were included.

Procedures
Once a scheduled C-section was reported, parents of babies who met all inclusion criteria were asked to provide their informed consent. Samples consisted of approximately 10 mL of amniotic fluid withdrawn with a syringe at the moment of the C-section, transferred to dropper vials that were then labeled and refrigerated.

A randomization procedure was implemented before the evaluations were made, for which each newborn infant was randomly assigned to one of the six groups resulting from the combination of three different doses (1, 5 or 25 drops) and three different intervals (at 1, 3.5 or 6 hours of life) (Table 1).

The evaluations were done in the incubators located in the mother and child rooming-in ward so that the effects of environmental stimulation on the response to the stimuli offered were controlled. Only the evaluation at 1 hour of life was performed in the delivery room because at this time the newborn infant was still in this area receiving the corresponding neonatal care. Amniotic fluid was administered at room temperature using cotton swabs that contained the corresponding number of drops. The stimulus remained at 2.5 cm from the baby’s nose for 15 seconds and then was removed for 45 seconds.

This was repeated five times. Two previous baseline minutes without stimulation were considered. General movements were recorded with a camera and used as a dependant outcome measure. Duration was analyzed by dividing the entire evaluation into 15 second intervals. For each interval, the beginning and ending of all body movements were timed. Three observers, who were blinded to the stimulation provided, analyzed the recording, but first a measure of interobserver reliability was established using a kappa coefficient, which resulted in 0.81.

Data analysis
The Statistica 6.0 software was used and mixed-design ANOVA tests were done for repeated measures considering average motor reactivity by group. The following outcome measures and their respective factors were included: time (1, 3.5 and 6 hours), dose (1, 5 and 25 drops), stimuli (1-5 minutes), and stimulation interval (0-15 seconds of stimulation; 16-30, 31-45 and 46-60 seconds post-stimulation). Post-hoc tests for Fisher’s least significant difference between means (alpha = 0.05) were used to study in greater detail the effect of factors or interaction locus between some of the factors.

For each subject we also did t tests for correlated samples comparing the means between the previous baseline and the stimulation period for each interval and dose.

RESULTS
In total, 65 consents were obtained but 36 were excluded due to problems related to amniotic fluid removal or because samples were contaminated with blood or meconium, and four were left out because the infants had respiratory distress.

Twenty five healthy newborn infants (13 girls and 12 boys) were studied, with a mean gestational age of 38.5 ± 1.20 weeks and a birth weight of 3216 ± 590 g.

The analysis of response at different times, regardless of the dose administered, showed that maximum reactivity was achieved during the 1st. hour, with a reduction at 3 hours and a recovery of reactivity at 6 hours (F[2.40] = 30.87; p < 0.0000). In addition, a maximum response was observed during the first minutes of stimulation (F[5.100] = 3.27; p < 0.0088) and, within such period, it was more significant during the first 15 seconds of stimulation (F[3.60] = 15.78; p < 0.0000), with a reduction observed in the rest
of the post-stimulation intervals (16-30, 31-45 and 46-60 seconds) (Figure 1).

T tests indicated that at the 1st hour of life, a dose of 1 drop of amniotic fluid resulted in a larger response when compared to baseline ($t=2.71; \ p \leq 0.026$). This occurred the first time the stimulus was offered (at 1 minute) and it was maintained as the dose was increased. When 5 drops were administered, the difference appeared at 1 minute ($t=2.40; \ p \leq 0.043$) and at 4 minutes ($t=3.09; \ p \leq 0.014$). When 25 drops were used, the difference was observed at 1 minute ($t=2.88; \ p \leq 0.027$). No significant differences were observed at 3.5 hours, except when 25 drops were administered ($t=2.49; \ p \leq 0.037$). At 6 hours, using 1 drop was again enough to show significant differences from baseline. This was particularly observed at 2 minutes ($t=2.36; \ p \leq 0.045$) and it repeated with higher doses. With 5 drops, differences appeared at 3 minutes ($t=2.50; \ p \leq 0.050$), while there was a hint of such differences at 1 minute with 25 drops ($t=2.21; \ p \leq 0.078$) and at 3 minutes ($t=2.13; \ p \leq 0.086$), but they were not significant (Figure 2).

**DISCUSSION**

The olfactory system is very important for the newborn infant to acquire information that will modulate his/her first behaviors.$^{10,11}$ Maternal odors trigger a sense of familiarity and are usually preferred over others.$^{12}$ This also occurs in preterm infants, which suggests an early trend to such cues.$^{13}$

In this study, newborn infants were stimulated with amniotic fluid in different doses and repeated at three post-natal intervals during their first hours of life. Newborn infants’ first response consists of a behavioral activation, which then gradually decreases. Response tends to go back to baseline levels once the odor is no longer present and it increases when each cotton swab is presented. The occurrence of this phenomenon in all tests would favorably support habituation processes and would allow to rule out motor fatigue or receptor adaptation processes. The potential detection of just one drop, even in the longest interval, would provide evidence in favor of familiarity with the stimulus during fetal gestation and its biological significance.

These results are only a preliminary approach to this subject and have certain limitations that interfere with their interpretation.

Such limitations include the reduced sample size because of the hurdles for obtaining enough clean samples of amniotic fluid and the small number of informed consents obtained from mothers undergoing scheduled C-sections.

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**Figure 1. Reactivity towards amniotic fluid at 1, 3.5 and 6 hours of life using stimuli (1-5 minutes) and stimulation intervals (0-15 seconds: with stimulation; 16-30, 31-45 and 46-60 seconds: post-stimulation)**

<table>
<thead>
<tr>
<th>General movements</th>
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<tbody>
<tr>
<td><img src="image" alt="Graph" /></td>
<td>At 1 hour</td>
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<tr>
<td><img src="image" alt="Graph" /></td>
<td>At 3.5 hours</td>
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<tr>
<td><img src="image" alt="Graph" /></td>
<td>At 6 hours</td>
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</tbody>
</table>

S: stimuli.
Figure 2. Reactivity towards different doses of amniotic fluid at different post-natal intervals with the five different stimuli used for evaluation.
This influenced the number of subjects for establishing a control group; instead, we used a baseline level for each subject. Given the protocol established for the removal of amniotic fluid, only infants born by C-section were included. However, based on prior studies, there are no differences in the olfactory response of infants born by C-section and those born by vaginal delivery.14

The present study opens a new pathway to preventive and therapeutic aspects. It is likely that smelling small amounts of amniotic fluid during hospitalization may be positive for establishing a more optimal ecological niche. Discussions are currently taking place regarding how sensory continuity of odors from prenatal life to postnatal life may influence the infant’s first adaptation responses in relation to self-regulation, emotional balance, feeding and mother-child interactions.15

CONCLUSION

Results seem to indicate that during the first hours of life, newborn infants are able to detect minimum doses of amniotic fluid and get accustomed to repeated stimulation. However, these findings need to be confirmed with a larger number of cases and by comparing groups exposed to amniotic fluid with others receiving a different olfactory stimulation.

REFERENCES


